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TRANSIENT BLOCKING OF PERSISTENT GNAWING BY HALOPERIDOL IN RATS WITH SEIZURE-INDUCED MULTIFOCAL BRAIN DAMAGE

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Summary

Frequent but phasic gnawing of objects is displayed by rats in which severe damage within: 1) the substantia nigra reticulata, 2) all of the amygdaloid nuclei except the central group and 3) multiple thalamic nuclei, was induced by lithium/pilocarpine-induced seizures. Multiple lesions were produced by these rats upon their own tails as well as upon the tails of their cage mates. These behaviors were considered predictable sequela of the disinhibition of compacta dopamine upon orofacial mechanisms that are modulated by the corpus striatum. In a manner similar to tail pinch-induced eating, the usually quick onset of spontaneous gnawing upon food chunks in a test setting was delayed transiently by appropriate dosages of haloperidol.

Orofacial dyskinesias are common correlates of insidious deterioration or damage within the nigrostriatal pathways of the mammalian brain (6). Persistent buccolingual-masticatory motions are major symptoms that emerge within a variety of clinical disorders (19). Experimental models for orofacial dyskinesias in the rat have emphasized teeth grinding and gnawing as the representative behavior (2, 17). The results of several paradigms (12) have suggested that these orofacial behaviors are mediated by the dopaminergic nigrostriatal pathway.

We (3, 5, 8, 10, 14, 15) have been documenting the neuro-morphological and behavioral sequelae that follow seizure-induction by a single subcutaneous (s.c.) injection of lithium (3 mEq/kg) followed 4 hr or 24 hr later by a single s.c. injection of 30 mg/kg of muscarinic agent pilocarpine (9). Normally these rats enter status epilepticus about 30 min to 40 min after the injection of the pilocarpine and die within 24 hr. However we have found that administration of the neuroleptic acepromazine (Atravet) reduces the mortality to less than 15% to 20%.

Because most of these animals survive, we have been able to measure subsequent changes in behavior that appear to be strongly correlated with insidious, multifocal neuronal loss (8, 15, 16). One domain of behaviors involves persistent teeth grinding and gnawing; qualitatively, they appear to be an exaggeration of the intensity and frequency of normal rodent mandibular behavior. When these rats are maintained singly or in groups, multiple small lesions have appeared upon their tails. Repeated observation has demonstrated that the tail lesions were induced by rats as they randomly encountered their own or another's tail.

Gnawing or biting responses are involved in many different classes of behaviors. Lammers, Kruk, Meelis, and van der Poel (11) differentiated various forms of orofacial activity by stimulating multiple sites within the hypothalamus. Teeth chattering, attack, aggressive social grooming and non-aggressive social grooming could be elicited during the stimulation of several, partial overlapping areas. The response area for social grooming was located almost entirely in the lateral hypothalamus. This area receives significant input from the central and lateral nuclei of the amygdala.

The specific amygdalohypothalamic connection is significant because of the conspicuous pattern of seizure-induced damage within the amygdala. Within 5 days of the seizure induction by systemic injections of lithium and pilocarpine, substantial neuronal drop out has been evident within all of the amygdaloid nuclei except the central group (15). Disinhibition of the

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dopaminergic component of the central nuclei because of the sudden seizure-induced devastation of the basolateral group (4) can facilitate the occurrence of dopamine-mediated behaviors.

The aberrant gnawing, which can be considered a subclass of orofacial dyskinesias, would be expected to occur for two reasons. First, the central nucleus directly innervates the rich dopamine-containing cells of the substantia nigra compacta (1) that then affect the dorsal striatum. Secondly, the seizures produced severe neuronal necrosis and gliosis within the reticulata; it normally inhibits the compacta. Iontophoretic dopaminergic stimulation of the substantia nigra attenuates the inhibitory responses of reticulata neurons in normal preparations (18). Consequently, endogenous hyperdopaminergic stimulation of an already compromised substantia nigra should significantly lower the threshold for gnawing behavior and contribute to its perseveration. From these observations, one logical hypothesis is that perseverative gnawing (secondary to disinhibited hyperdopaminergic compacta activity because of reticulata damage) should be diminished transiently by a dopamine (D2) blocker.

To test this hypothesis we selected a task that is sensitive to dopaminergic stimulation. Brief mechanical stimulation (pinching) of the tail induces non-appetitive gnawing that precedes eating; the behavior is blocked by therapeutic dosages of haloperidol (2). The increased turnover and release of dopamine within the striatum (but not in the frontal cortex, hippocampus or olfactory tubercle) after a 2-min tail pinch (12) supports the hypothesis that the general domain of orofacial behaviors may be mediated by the dopaminergic nigrostriatal pathway.

These predictions are considered logical extrapolations of the concept developed by Fibiger and Lloyd (6); these researchers have argued that many kinds of orofacial dyskinesias, including the tardive buccolingual-masticatory syndrome, are associated with disruption of GABA-containing neurons. Whereas their model implicated slow, drug-induced damage of these neurons within the striatum and hence disruption of the feedback to the nigral reticulata, we suspected that relatively sudden seizure-induced damage within this structure simulated the loss of GABA striatal neurons.

General Observations and Characteristics of the Phenomenon

During the last three years approximately 100, 5 minute observation periods have been completed. Audible teeth grinding, which is indiscriminable (just more intense and longer) from the behavior displayed by normal rats during perisleep episodes, has been observed in about 50% of the time. Gnawing of objects, which becomes conspicuous about 7 to 10 days after the induction of the seizures, always displayed a stereotyped sequence. The rat slowly moved about the cage; invariably there was exaggerated nasal and vibrissae movements. When an object was encountered, the rat bit it; the first bite was forceful while the subsequent bites were less intense and appeared to be stereotyped continuations.

When food-deprived rats were tested within a radial arm maze, some animals bit the food dish (a small cap) with such intensity that an audible pop was heard throughout the room. Only the first bite was audible and in about one-third of these animals there was transient extension of the neck and of all four limbs. Within the home cage, non-food-deprived rats gnawed food cubes (on the floor of the cage) or a tail in a similar manner. As the other rat moved away, the gnawing rat followed the tail and chewed it until either the other rat displayed a defensive stance or another rat walked between the two animals. When housed singly or after a cagemate had been removed because the tail has been chewed, these rats gnawed their own tails if the tail, after the rat rotated its body, remained near its mouth. The incidence of gnawing increased after about postseizure day 10 and continued for at least two months.

Approximately 60% to 70% of rats (sample size of 300) in which seizures were induced displayed the type of tail damage that is shown in Figure 1. In about 10% of the cases, the number of micropunctures were so extreme that the tail lost its tone; approximately 10% of these rats ultimately lost the distal one-third of their tails. Of the 150 to 200 fights (as defined by rearing and boxing positions followed by an attack that drew blood), more than half were

preceded by persistent tail gnawing or excessive grooming. Consequently these rats often displayed torn ears or multiple scars on their faces. When 3 rats were housed per cage, removal of at least one rat for its own safety and for humane reasons (Canadian Council for Animal Care Guidelines) occurred for every 8 cages.

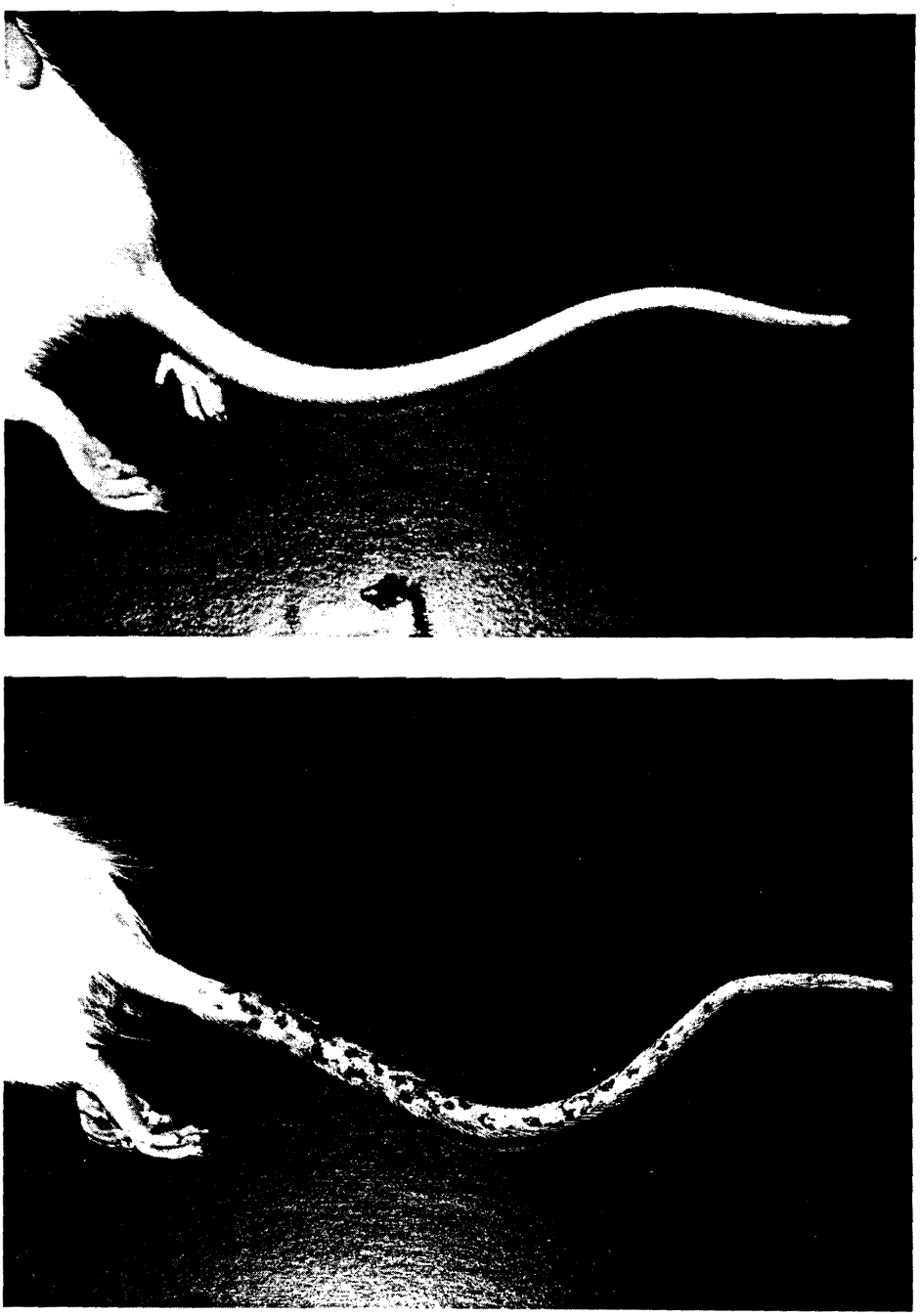

FIG. 1
(A: top) normal rat tail; (B: bottom) tail of rat that had been housed with rats in which persistent teeth grinding and gnawing emerged within a few days after seizures that were precipitated by a single, subcutaneous injection of lithium (3 mEq/kg) and pilocarpine (30 mg/kg).

METHODS

Seizure-induction

There were two separate experiments. In Experiment I, 18, 100 to 110 day old female Lewis rats served as subjects; each rat was injected with a single subcutaneous (hind flank) injection of 3 mEq/kg of lithium chloride (3 mEq/cc) and then 24 hr later with a sub. cu. injection of pilocarpine (30 mg/kg). After the display of standing and forepaw clonus, each rat was injected (sub cu) with 25 mg/kg of acepromazine (Atravet from Ayerst Laboratories, Montreal). In Experiment II, the same procedure was employed to induce seizures in 14, 100 to 110 day old female Lewis rats. An additional 12 rats served as normal controls. Throughout the experiment, the rats were housed 3/cage within a room that was temperature controlled (22°C) and light-dark (12:12; onset 0730 LT) regulated; food and water were available ad libitum.

Testing Procedure

In both experiments, each rat was tested singly. The rat was placed in a 37 cm by 30 cm by 18 cm (deep) white plastic container. Its floor was marked into 4 equal parts; 6 or 10 (there were no group differences so this variable was collapsed) normal-sized pieces of Purina rat chow were scattered evenly on the surface. After the rat was placed in the box: 1) the time before gnawing of the most proximal rat chow, and 2) the numbers of squares traversed (Experiment I), were recorded. Total field exposure was 2 min; the rat was then returned to its home cage.

In Experiment I, 5 to 7 days after the seizure induction, each rat was first tested in the food box and then assigned randomly to one of three groups (6/group): saline, 0.2 mg/kg of haloperidol [Haldol (injectable) from McNeil Pharmaceutical, Stouffville, Ontario, Canada] and 0.4 mg/kg of Haldol; the dosages were selected in light of the results of Antleman and Szechtman (2); appropriate treatments were administered by intraperitoneal (i.p.) injections. One hour, two hours and then again 24 hr after the injections, each rat was again tested in the food box. In Experiment II, 10 to 15 days after the seizure induction, the latency to gnawing was determined for each rat. All of the rats in which seizures had been induced (n=14) as well as the normal animals (n=12) then received 0.4 mg/kg of Haldol (i.p.); their latencies were determined again 30 min, 60 min and 24 hr later.

Experimental Design

Because the normal (no seizure) rats never gnawed, i.e., latencies >120 sec, these rats were not used in subsequent analyses. In Experiment I, the differences between the time (in sec) required to initiate gnawing one hour and two hours after the treatment (saline; two dosages of Haldol) was administered, were subtracted from each rat's baseline value. Similar scores were determined for the activity measures, which were used as an index for the possible artifacts of drug-induced lethargy, for each rat. One way analyses of variance by group (treatment) for the relative latency (observed minus baseline) and for the activity were determined; Tukey's (set at $p < 0.05$) was used as the post hoc procedure. In Experiment II, correlated t-tests were completed between the baseline latency to gnaw and these latencies +30 min and +60 min after the drug injection. Differences scores (subtractions from baseline values) for each rat were employed in order to minimize the contribution of individual differences. T-tests rather than repeated measures analyses were completed because of their simplicity and because of the significant disparity in variances that normally accompany any large changes in means between treatments). All analyses involved SPSSX software on a VAX 4000 computer.

Brain histopathology. Within a few days after the termination of the experiment, brains were removed and fixed in E.F.A. for 48 hr. The cerebrums were processed, sectioned (10 μm) and stained with toluidine blue O as described previously (13). In order to insure that the pattern of brain damage was similar to other studies (15), two brains were examined, between the mesencephalon and rostral telencephalon at 400 X according to previous procedures.

RESULTS

The latencies to initiate gnawing of food chunks within the test box, relative to the pre-drug baseline trial, for the epileptic rats that had received the saline and Haldol treatments are shown in Figure 2. One way analyses of variance demonstrated significant group differences one hour [$F(2,15)=7.34$, $p<0.001$] and two hours [$F(2,15)=5.07$, $p<0.01$] after the drug treatment. Post hoc analyses showed that the major source of the variance was due to the increased delay of gnawing latency for the group that received the 0.4 mg/kg of Haldol relative to those who received 0.2 mg/kg of Haldol or saline; the latter did not differ significantly from each other. By 24 hr after the injections, the group differences [$F<1.00$, $p>0.05$] had disappeared; the means and standard deviations for those latencies (relative to baseline) for the saline, 0.2 mg/kg and 0.4 mg/kg groups, were -2(8) sec, 6 (12) and -10 (20) sec, respectively. There were no statistically significant treatment differences [$F(2,15)<1.00$, $p>0.05$] with respect to general activity during the testing.

In Experiment II, the means and standard deviations for the onset of gnawing times for the baseline trial and for each trial 30 min, 60 min and 24 hr after the injection of 0.4 mg/kg of Haldol were 7.9 (7.7) sec, 55.1 (37.5) sec, 23.1(20.6) sec and 5.8 (8.1), respectively. Correlated t-tests demonstrated statistically significant increases in gnawing onset 30 min [$t(13)=4.77$, $p<0.001$) and 60 min ($t=2.46$, $p<0.05$) after the injection but not 24 hr ($t<1.00$) after the injection of the dopamine-blocking agent; the decrease in latency between 30 min and 60 min after the Haldol was also significant [$t(13)=2.96$, $p=.01$]. In both experiments, the gnawing, once initiated, was a persistent and semi-continuous process. Normal rats, in which seizures had not been induced, **never** gnawed upon the food cubes during the 2 min test periods.

Histopathological evaluation of the brains of the rats in which the seizures had been induced indicated a pattern of damage that was identical to the other brains from rats that had been killed between 5 and 20 days after the induction of the seizures. The major areas of damage (neuronal drop out) within the limbic region included all of the amygdaloid nuclei except for the central group, CA1 of the hippocampus and the entorhinal/pyriform cortices. Within the thalamus, the major damage occurred within the mediodorsal thalamus, the posterior thalamic group, the parafascicular nucleus and the suprageniculate nucleus. The only area that displayed damage within the mesencephalon was the reticulata component of the substantia nigra; an example of the typical pattern of neuronal drop out and gliosis is shown in Figure 3.

DISCUSSION

Normal rats, that are maintained on food and water ad libitum, do not gnaw upon food blocks within an open field. The persistent occurrence of this behavior in rats in which brain damage had been induced by lithium/pilocarpine-precipitated limbic seizures, indicates that inhibitory mechanisms had been disrupted. Qualitatively there was no clear distinction between normal masticatory responses and those displayed by the rats that exhibited the conspicuous spontaneous teeth grinding and the perseverative gnawing. These quantitative increases suggest disinhibition of dopamine-mediated orofacial mechanisms.

The hypothesis was supported that haloperidol [at a dosage sufficient to block tail pinch induced gnawing (2), without producing generalized lethargy] transiently blocks the persistent gnawing in rats in which the substantia nigra reticulata had been damaged. That the delay in the onset of gnawing was not confounded by reduced locomotor activity or responsiveness was indicated by the absence of significant group differences in ambulation within the food box.

Whereas 0.2 mg/kg of Haldol was found to delay the onset of tail pinch-induced eating in normal rats, this dosage was not effective in the present study. However, in light of the severity of the reticulata damage evoked by this seizure model, one would expect relatively greater maintained elevations in striatal dopamine release than would be present following the brief tail pinch procedure. The behavioral correlate would be gnawing and eating latencies that were similar to rats that had not been treated by this dopamine-blocking neuroleptic.

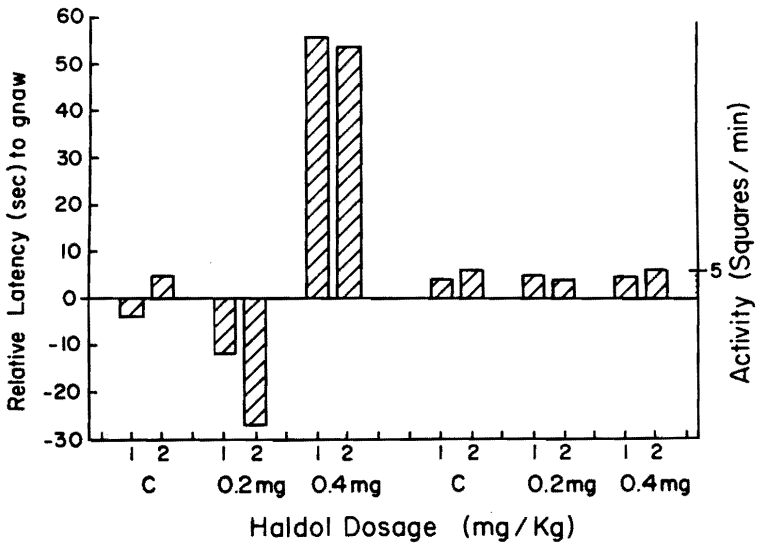

FIG. 2
A) Left axis and left half of graph: relative latency to initiate gnawing of food chunks within a small open field one or two hours after injection of haloperidol or saline [S.E.M.s for drug treatments between 14.8 and 25.5 sec] B) Right axis and right half of graph: numbers of squares traversed per minute during the 120 sec exposure to the test field.

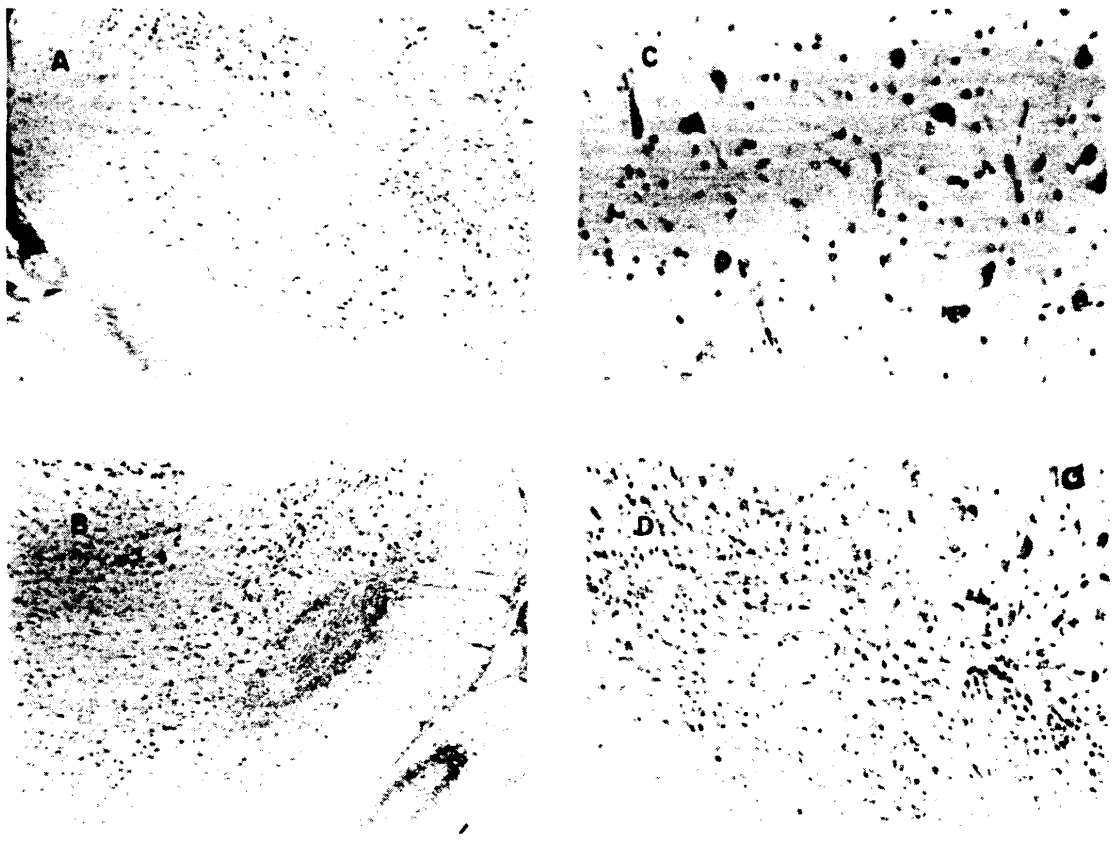

FIG. 3
Photomicrographs of the substantia nigra of the rat. A) normal rat, 40X; B) selective damage within the reticulata of a rat in which seizures had been induced by peripheral injections of lithium and pilocarpine, 40 X; C) 400 X of the reticulata region from a normal rat; D) 400 X of the reticulata region of a rat in which seizures had been induced about one week previously; note cell drop out and gliosis. All sections were stained with toluidine blue O.

The results suggest that the more frequent gnawing displayed by these rats in the food box is related to the same neuropathway as tail-pinch induced teeth grinding, chewing and eating. Rats in which limbic-like seizures had been induced frequently displayed loud and conspicuous masticatory movements within 10 to 20 sec after thermal and mechanical stimulation of the distal third of the tail [while blood was being drawn from the tail vein (K. Reid, unpublished observations)]. Because the rat is restrained during this procedure, it gnaws on the most proximal object, e.g., the surrounding plastic container.

The relatively weaker effect of 0.4 mg/kg of Haldol in Experiment II was indicated by the decay in the latency of the gnawing that was evident after only one hour. In Experiment I, the blocking effect was still apparent two hours after the Haldol injection. This discrepancy could have reflected the differences in post-seizure time between the two groups. Whereas the rats in Experiment I were tested 5-7 days after the seizure induction, the rats in Experiment II were tested 10-15 days after the induction. Because the cell loss within the reticulata continues after the seizure (for at least 2 months at which time an asymptote appears to be reached), one would expect a greater net disinhibition of the compacta, release of dopamine, persistent gnawing and less effectiveness over time of a systemically applied neuroleptic in the second experiment.

The results of the two experiments are commensurate with our general hypothesis concerning the consequences of lithium/pilocarpine-induced seizure damage. We (3) have suggested that the effects of massive cholinergic stimulation and subsequent excitotoxic damage within most (and especially the basolateral nucleus) of the amygdala release the (never damaged) dopaminergic central nucleus from inhibitory control. All structures that are strongly innervated by the central nucleus would be stimulated and their correlative behaviors would dominate the animals' profiles. Their short latency for persistent gnawing of food cubes, similar in both profile and duration to the behaviors evoked by tail-pinch, would reflect the chronic elevation in dopamine stimulation.

The unusual tail damage and social behavior that begins to emerge about one week after the induction of the seizure could also be explained by the disinhibition of the central amygdaloid nucleus. It projects to the lateral hypothalamus that has been strongly implicated in social grooming (11). Unusual phasic stimulation of the lateral hypothalamus could activate these behaviors and initiate the behavioral sequence that leads to the tail gnawing. The elevated nociceptive (25% to 30% increase) threshold (15) for these rats, which would be compatible with the observed damage in the parafascicular nuclei of the thalamus, could contribute to the persistence of the gnawing until it reached some critical level.

Involvement of the lateral hypothalamic "sequences" is also suggested by the time-dependent emergence of the tail gnawing behaviors; they emerge after the first week following the induction of the seizure (15). If we assume that quantitative increases in neuronal drop-out are preceded by excessive neuronal activity, then the specific loss of neurons within the lateral amygdaloid nuclei between post-seizure day 5 and day 15 suggests transient stimulation of the areas that are innervated by this nuclear group. The lateral basal nucleus also projects to the lateral hypothalamus.

The central nucleus is also the major amygdaloid source of efferents to the substantia nigra (pars compacta), the central grey, parabrachial nuclei, locus ceruleus and parasolitary groups. However, with the exception of the substantia nigra reticulata, we have never observed any discernable damage within or below the mesencephalon. The disinhibition of the activity of the compacta by the conspicuous damage to the reticulata would be expected to not only enhance the endogenous dopamine release to the nigrostriatal target areas but to amplify this release because of the abnormal stimulation from the central amygdaloid nucleus. That dopamine disinhibits the control of reticulata neurons upon the adjacent compacta is well established (18).

The substantia nigra reticulata has been considered a final gateway that prevents the spread of epileptiform activity into overt motoric automatism (7). Several types of seizure-inducing techniques damage this structure (7). Considering the paroxysmal-like features of the orofacial dyskinesias that are displayed by rats in which lithium/pilocarpine seizures have been induced,

perhaps insight into the mechanisms of this syndrome within clinical populations might emerge if it is considered a variant of periodic but focal epileptiform discharges.

Fibiger and Lloyd (6) have challenged the prevailing explanation that dyskinesias are associated with functional increases in dopamine efficacy (either by receptor supersensitivity or enhanced transmitter release) within the terminal branches of the nigrostriatal pathway. They argue that prolonged use of neuroleptic drugs produced specific neuronal loss within the ventrolateral region of the striatum; it is concerned with the innervation of the oral musculature. Damage to these neurons would disrupt the GABA inputs to the globus pallidus as well as to the substantia nigra (reticulata). GABA receptor antagonists injected into the substantia nigra are known to precipitate oral dyskinesias in rats (6).

Our results can be interpreted as support for Fibiger and Lloyd's model. The inevitable, seizure induced damage within the substantia nigra reticulata would be the functional equivalent of cell loss within the ventrolateral striatum. The consequence of the loss of GABA innervation from the striatum or the loss of GABA-receiving cells within the reticulata would be the release of dopamine from synthesizing cells within the adjacent compacta. This disinhibited activity would facilitate neuronal function (via the nigrostriatal pathway) within the striatum where orofacial mechanisms are modulated. The net effect would be a disinhibition of masticatory activity that is typically elicited only during transitional arousal states and food consumption.

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